Allelic Variants of Drug Metabolizing Enzymes as Risk Factors in Psoriasis

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The onset or exacerbation of psoriasis, a T-cell-dependent skin disease with autoimmune features, can be triggered by drugs such as antimalarials and beta-blockers. Xenobiotics may also play a role in idiopathic psoriasis. It has been hypothesized that different metabolic efficiencies caused by variant alleles of xenobiotic metabolizing enzymes could lead to the accumulation of xenobiotics or their reactive metabolites in target organs. Subsequently, neoantigens or cryptic peptides could be presented and initiate an aggressive T cell response. In this context, we analyzed a broad array of xenobiotic metabolizing enzymes in up to 327 Caucasian psoriasis patients and compared them to 235 control persons. Alleles tested include four phase I and three phase II enzymes. Significantly more carriers of the variant alleles of *CYP1A1* (alleles **2A* and **2C*) were found in healthy controls than in patients, suggesting a protective role for these alleles. No significant difference between patients and controls could be found, however, for the other phase I alleles *1B1 *1* and *1B1 *3*, *2C19 *1A* and *2C19 *2A*, and *2E1 *1A* and *2E1 *5B*. Of the variant alleles coding for phase II enzymes only *GSTM1*, but not *GSTT1* or *NQOR*, correlated with a risk to contract psoriasis. Some combinations of phase I and phase II enzymes suggested protective or risk-associated effects. Interestingly, heterozygosity for *CYP2C19* alleles **1A* and **2A* was associated with increased risk for "late onset" psoriasis, whereas this genotype was protective for psoriatic arthritis. This is the first large-scale study on these enzymes and the results obtained support the concept that different activities of metabolizing enzymes can contribute to disease etiology and progression. ***Key words: autoimmunity/human/immunotoxicology/susceptibility genes/xenobiotic metabolism. J Invest Dermatol 120:765–770, 2003***

Psoriasis, affecting about 2% of Caucasians, is an idiopathic chronic inflammatory disease of the skin with autoimmune features. Psoriasis is characterized by a marked hyperproliferation of keratinocytes in association with vascular expansion, leukocyte infiltration, and altered cytokine production (Valdimarsson *et al*, 1986; Kapp, 1993; Ortonne, 1996). Psoriasis has a genetic basis, although the mode of inheritance (e.g., dominant/recessive) remains unclear. Concordance in monozygotic twins is not absolute with 63%–70%, pointing to environmental factors in addition to genetic factors in the onset and severity of the disease (Farber *et al*, 1974; Bhalerao and Bowcock, 1998). Reports on associations with environmental components include infections, endocrine factors, and drugs. Lithium, antimalarials, beta-blockers, ACE inhibitors, nonsteroidal anti-inflammatory drugs, terbinafine, cigarette smoking, and heavy consumption of alcohol have been found to provoke or exacerbate psoriasis, although evidence is often anecdotal and not epidemiologically secured (Rosenberg and Belew, 1982; Abel *et al*, 1986; Kruger and Duvic, 1994; Baker, 2000). With respect to autoimmune diseases the impact of environmental factors such as drugs, infections, and others is conceivably mediated by direct or indirect effects on compartments of the immune system, which may result in *de novo* immunogenicity of self-proteins (Griem *et al*, 1997). Chemical compounds or their metabolites can become immunogenic in two ways: either they can attach to a peptide and be presented along with that peptide, or by binding to cellular proteins they can change the normal breakdown of those proteins in antigen processing, leading to the presentation of cryptic peptides and subsequent T cell activation. An example of the former mode, leading to allergic drug reaction, is presentation of penicillin to T cells (Weltzien and Padovan, 1998). Protein-reactive oxidation forms of gold salts represent an example of the latter (Griem *et al*, 1996), where new self-antigens are generated and become available against which the immune system is not tolerant. Adverse immune reactions to self, e.g., unresolvable inflammation, can then be the consequence. Xenobiotic metabolizing enzymes (XMEs) play a central role for the occurrence of neoantigens because they control the breakdown pathways and kinetics for the metabolism of endogenous and exogenous chemical substances. In humans, allelic forms of many XMEs are known, often resulting in changed efficiencies of that particular XME. In consequence, this may result in genetically controlled preferential generation of protein-reactive metabolites or a longer persistence (and thus availability) of a protein-reactive

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Abbreviations: PsA, psoriatic arthritis; RefS, reference sequence; VarS, variant sequence; XME, xenobiotic metabolizing enzyme.

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substance in the body, which conceivably can translate into immunogenicity of that substance. For instance, this has been shown in man and mice where slow acetylators easily elicit an immune response against procainamide, whereas fast acetylators are less susceptible (Uetrecht and Woosley, 1981; Wolkenstein *et al*, 1995).

Small chemical compounds like the drugs mentioned above are conspicuous in eliciting or exacerbating psoriasis and have been suggested to play a role in the T cell response (Rosenberg and Belew, 1982; Halevy and Livni, 1993). By analyzing allelic variants of a wide range of XMEs in a large group of patients suffering from psoriasis we therefore wanted to test the hypothesis that the genetic setup of XMEs is linked to psoriasis.

MATERIALS AND METHODS

Study population Three hundred and twenty-seven psoriatic patients attending dermatology clinics in Düsseldorf, Bad Bentheim, and Bad Salzschlirf, all in Germany, participated. Only a very small number of the patients that we approached reneged. Two hundred and thirty-five healthy control subjects were recruited from voluntary blood donors in Frankfurt/Main, Germany, and through an unrelated study in Dortmund, Germany. Written informed consent was obtained from all subjects. The study was approved by the ethical committee at the University of Düsseldorf. All subjects were Caucasians (here, Germans). Genomic DNA was isolated from 5 ml blood samples with a commercial isolation kit (Boehringer, Mannheim, Germany).

Genotyping for reference sequences and allelic variants We use the terms reference sequence (RefS) and variant sequence (VarS) throughout this report. Usually the reference sequence is the first allele described or the most common one.

Cytochromes P450 *CYP1A1* **2A*, *CYP1B1*, *CYP2C19*, and *CYP2E1*, and *NQOR* (synonyms: DT diaphorase, quinone oxidoreductase), and the glutathione-S-transferases *hGSTM1* and *hGSTT1* were typed on the genomic DNA. With the exception of the glutathione-S-transferases, allelic variants were identified by restriction fragment length polymorphism after appropriate restriction enzyme digestion of the polymerase chain reaction (PCR) fragment (RFLP-PCR). Positive controls with DNA of known genotype were included each time, and random samples were typed several times. **Table I** lists primer sequences, PCR conditions, and restriction digest enzymes.

The *GSTM1* and *GSTT1* alleles were analyzed by multiplex PCR as described elsewhere (Wiebel *et al*, 1999), and depending on the presence or absence of the respective DNA bands the study subjects were categorized as homozygous null (VarS) or carrier of the gene (RefS). The *CYP1A1* allele **2C* was analyzed in a LightCycler (Roche™; Wittwer *et al*, 1997). Outer primers were used to amplify the gene, and fluorescent primers covering the site of base exchange were added. The melting curves then identify RefS and VarS. LightCycler primer sequences are given in **Table II**.

Nomenclature The following nomenclature was used to describe the different polymorphic variants (Garte *et al*, 2001a; Ingelman-Sundberg *et al*, 2001). For the *CYP1A1* polymorphisms the genotype is *CYP1A1* **1A/*1A* for the reference sequence. The *CYP1A1* **2A* allele has a 3801T > C exchange (allele also known as Msp); *CYP1A1* **2C* allele has a 2455A > G exchange (allele also known as IVA). *CYP1A1* **2B* is the name of the allele if both of these exchanges are at the same locus; we did not determine this allele here. For the *CYP1B1* locus allele the reference allele is called **1*, and the 4326C > G (Val432Leu) polymorphism is called **3*. For *CYP2C19* and *CYP2E1* the reference allele is called **1A*, and the polymorphic variants are called **2A* (681G > A) and **5B* (–1293G > C and –1053C > T), respectively. The *GSTM1* **1/*1* and *GSTM1* **1/*2* are referred to as carriers, with *GSTM1* **2/*2* genotype used to indicate the homozygous null variant. Similarly, the *GSTT1* carriers include the genotypes *GSTT1* **1/*1* and **1/*2*, with the *GSTT1* **2/*2* genotype indicating the deleted phenotype.

Statistics Allele frequencies were calculated according to the Hardy–Weinberg equilibrium. The two-sided Fisher's exact test was used to test contingency tables for significant associations between patient groups and control groups for the various enzymes as indicated in *Results*. Odds ratios (OR) and 95% confidence intervals (95%CI) were calculated to measure the strength of the observed associations. All statistical calculations were done with Prism 3.0 software (GraphPad, San Diego, CA). Where indicated the Bonferroni post test was used to adjust for multiple comparisons: if k tests were done, the result of each test was considered significant if $p < 0.05/k$, thus ensuring an overall significance level of 5%.

RESULTS

Subjects The study group consisted of 327 patients and 235 controls. All patients and controls were typed for all enzymes indicated, although due to experimental losses in some cases the absolute number of typed subjects varies slightly for the

Table I. Conditions of RFLP-PCR

Gene	Primer[a]	PCR profile[b]	RFLP enzyme[c]	Name of variant allele
CYP1A1	5′ > CAGTGAAGAGGTGTAGCCGCT < 3′	55°C, 30″	MspI	*2A (Msp)
[ref. 1][d]	5′ > TAGGAGTCTTGTCTCATGCCT < 3′	72°C, 1′		
		35 cycles		
CYP1B1	5′ > AAC GTC ATG AGT GCC GTG TGT < 3′	57°C, 1′	BfrI	*3 (G)
[2][d]	5′ > GGC CGG TAC GTT CTC CAA ATC < 3′	72°C, 1′		
		38 cycles		
CYP2C19	5′ > AAT TAC AAC CAG AGC TTG GC < 3′	58°C, 1′	SmaI	*2A (m1)
[3][d]	5′ > TAT CAC TTT CCA TAA AAG CAA G < 3′	72°C, 1′		
		35 cycles		
CYP2E1	5′ > CCC GTG AGC CAG TCG AGT < 3′	61°C, 1′	RsaI, PstI	*5B (C2)
[4][d]	5′ > ATA CAG ACC CTT TTC CAC < 3′	72°C, 1′		
		30 cycles		
GSTM1[e]	5′ > GAACTCCCTGAAAAGCTAAAG	60°C, 1′		Homozygous absence of enzyme (null allele)
[5][d]	5′ > GTTGGGCTCAAATATACGGTGG	72°C, 1′		
		35 cycles		
GST T1[e]	5′ > TTCCTTACTGGTCCTCACATCTC	60°C, 1′		Homozygous absence of enzyme (null allele)
[6][d]	5′ > TCACCGGATCATGGCCAGCA	72°C, 1′		
		35 cycles		
NQOR	5′ > ATTTGAATTCGGGCGTCTGCTG < 3′	58°C, 1′	HinfI	Ser187 (null allele)
[7][d]	5′ > GAGACGCTAGCTCTGAACTGAT < 3′	72°C,1′		
		30 cycles		

[a]Primers used for PCR are listed as forward primer (upper line) and reverse primer (second line). Primer sequences were taken from the literature.
[b]Annealing temperature and duration/extension temperature and duration/number of cycles. A denaturation step at 95°C for 1 min preceded each cycle.
[c]Enzyme used to cut for determination of restriction fragment length polymorphism indicative of variant allele.
[d][1] Hayashi *et al* (1991); [2] Bailey *et al* (1998); [3] de Morais *et al* (1994); [4] Persson *et al* (1993); [5, 6] Wiebel *et al* (1999); [7] Eickelmann *et al* (1994).
[e]In order to verify DNA amplification in the assay, β-hemoglobin was co-amplified (data not shown).

individual enzymes. Age distribution and gender were similar for both patient and control group (**Table III**, ***A***). Approximately two-thirds of the patients had suffered an early onset of disease, with women showing an earlier onset (mean at age 19 for female patients *versus* mean at age 22 for male patients; mean age for late onset was 55 and 52 y, respectively). Twenty-seven men and 22 women patients suffered also from psoriatic arthritis (PsA). Diagnosis was confirmed by X-ray and/or scintigraphy. The gender ratio of this subgroup of patients was no different from that of patients suffering from classical psoriasis or healthy controls (**Table III**, ***B***). Approximately one-third of the patients reported a familial occurrence (first-degree relatives) of the disease. No statistically significant difference could be detected between men and women or psoriasis vulgaris and PsA in this respect (**Table III**, ***B***).

Table II. Primers for LightCycler

Gene	Primer[a]	Name of variant allele
CYP1A1[b]	5′ > GAACTGCCACTTCAGCTGTC < 3′ 5′ > ACCAGACCAGGTAGACAGAGTC < 3′ sensor: GAAGTGTATCGGTGAGACCGTTICC X anchor: CTGGGAGGTCTTTCTCTTCC TGGCTATC	*2C (IVA)

[a]Primers used for Lightcycler PCR are listed as forward primer (upper line) and reverse primer (second line) plus sensor and anchor probe. Primers and PCR conditions were designed/calculated by the company TIB MOLBIOL, Berlin, Germany.
[b]See Hayashi *et al* (1991).

***CYP1A1* variant alleles **2A* and **2C* are protective factors for psoriasis** In order to test for the hypothesis that genetic variants of XMEs are associated with risk of psoriasis, we determined the number of homozygous/heterozygous carriers of a panel of phase I and phase II XMEs in psoriasis patients from Germany and compared them to control persons. Genes were chosen that cover several phase I and phase II enzymes, had been suspected as relevant before, and include those enzymes that might be relevant in the metabolism of alleged psoriatogenic drugs.

The number of carriers of a certain allele combination (homozygous or heterozygous) was counted and analyzed using contingency tables. The statistical evaluation of differences between patients and controls is compiled in **Tables IV, V, and VI**. No significant differences between patients and controls could be observed for any phase I enzyme, with the exception of *CYP1A1* (see Tables IV and V). *CYP1A1* is involved in the hydroxylation of polycyclic aromatic hydrocarbons such as benz[a]pyrene. The enzyme is inducible by, for example, cigarette smoke or cruciferous vegetables. Several polymorphic variants have been identified; we analyzed two, which result in increased activity of the enzyme. The *CYP1A1* reference sequence is referred to as **1A*. The rare variant allele *CYP1A1 *2C (*allele frequency of approximately 2%), responsible for a higher activity of *CYP1A1*, has a single nucleotide exchange at nucleotide 2455 that results in an isoleucin to valine exchange (Hayashi *et al*, 1991). We also typed patients and controls for a second variant allele of *CYP1A1*, the **2A* allele. This allele has a T3801C exchange (Petersen *et al*, 1991), resulting in easier induction of the gene. The nucleotide exchanges resulting in alleles **2A* and **2C* can be found alone or together in the gene locus; the latter case is then called **2B*. The risk potential of *CYP1A1* alleles **1A*, **2A*, and **2C* is shown in Tables IV and V.

Table III. Study population

A	Men (%)	Women (%)	Mean age men[a]	Mean age women	
Patients	164 (50.3%)	162 (49.8%)	48.5 (14.4)	48.7 (15.5)	
Controls	130 (57.0%)	98 (43.0%)	47.6 (13.1)	45.3 (13.5)	
	n.s.		n.s.		
B	PsV (men)[b]	PsV (women)	PsA (men)	PsA (women)	
Onset age[c] < 40	67 (82%)	56 (79%)	25 (93%)	17 (77%)	n.s.
Onset age > 40	15	15	2	5	n.s.
Familial association[d]	30 (34%)	34 (29%)	15 (52%)	10 (43%)	n.s.
No familial association	58	49	14	13	n.s.

[a]Mean age and SD (in parentheses) of indicated subjects in years.
[b]PsV, patients diagnosed with psoriasis vulgaris; PsA, patients diagnosed with psoriatic arthritis (including those who had PsA *and* PsV).
[c]Number of patients where disease had started when they were under the age of 40, or over the age of 40.
[d]Number of patients who reported next of kin to have psoriasis, too.
n.s., not significant.

Table IV. Genotype distribution for CYP1A1 reference sequence *1A and variant sequences *2A and *2C

Genotype[a]	Patients	Controls	Exact p-value	OR (95%CI)[b]
CYP1A1 *1A/*1A	272 (88%)	176 (76.8%)	p = 0.0006	2.28 (1.431–3.635)
*CYP1A1 *1A/*2C*	0	16	p < 0.0001	–
*CYP1A1 *2C/*2C*	0	1	n.d.	–
*CYP1A1 *1A/*2A*	25	36	p = 0.0086	0.44 (0.27–0.81)
*CYP1A1 *2C/*2A* or **2B*[c]	9	0	n.d.	–
*CYP1A1 *2C/*2B*	1	0	n.d.	–
*CYP1A1 *2A/*2A*	0	0	n.d.	–
*CYP1A1 *2B/*2A* or **2B/*2C*[c]	1	0	n.d.	–
*CYP1A1 *2B/*2B*	0	0	n.d.	–

[a]Two single nucleotide exchanges at different positions in the CYP1A1 gene, i.e., alleles **2A* and **2C*, were typed for patients and controls. Numbers of individuals carrying any of the nine possible allele combinations were counted. The reference sequence *CYP1A1 *1A* has no mutations (see *Materials and Methods* for nomenclature).
[b]Fisher's exact test on contigency tables of the numbers of carriers of this genotype *versus* the sum of all the others. OR and 95%CI. n.d., not done, as genotype too rare.
[c]For this combination it is not possible to distinguish with our method of typing the alleles in two independent assays whether or not the nucleotide exchanges are on the same chromosome or not.

TableV. Genotype distribution of the described XMEs in patients and control individuals

	Patients			Control individuals				
Enzyme[a]	Homozygous RefS	Heterozygous	Homozygous VarS	Homozygous RefS	Heterozygous	Homozygous VarS	p-value	OR[b] (95%CI) of VarS
CYP1A1 *1A/*2A or 2C	272 (88.3%)	36 (11.7%)	0 (0%)	176 (76.8%)	53 (23.1%)	1 (0.4%)	0.0004*	0.44 (0.27–0.68)
CYP1B1 *1/*3	93 (29.5%)	146 (46.3%)	76 (24.1%)	75 (31.9%)	113 (48.1%)	47 (20.0%)	0.571	1.12 (0.78–1.63)
CYP2C19 *1A/*2A	235 (73.6%)	75 (23.5%)	9 (2.8%)	163 (69.6%)	66 (28.2%)	5 (2.1%)	0.338	0.82 (0.56–1.19)
CYP2E1 *1A/*5B	306 (95.6%)	14 (4.3%)	0	222 (94.4%)	12 (5.1%)	1 (0.4%)	0.554	1.28 (0.59–2.77)
GST M1	131 (49.1%)	136 (50.9%)		89 (40.3%)	132 (59.7%)		0.055	0.70 (0.48–1.00)
GST T1	206 (77.4%)	60 (22.6%)		184 (80.0%)	46 (20.0%)		0.511	1.16 (0.75–1.79)
NQOR	209 (66.8%)	91 (29.1%)	13 (4.1%)	169 (72.2%)	52 (22.2%)	13 (5.6%)	0.191	1.29 (0.89–1.87)

[a]Enzymes with known polymorphisms were genotyped for approximately 300 psoriasis patients and approximately 230 controls. See *Materials and Methods* for details. Number of carriers either homozygous for the RefS, heterozygous for RefS and VarS, or homozygous for VarS are shown. Genotype frequencies are given in parentheses.
[b]OR for the carriers of VarS (heterozygote plus homozygote) in patients *versus* controls was calculated by Fisher's exact test. 95%CI were calculated with GraphPad Prism™ using the approximation by Katz *et al* (1978). Probability values p and ORs are shown.
*Significant after Bonferroni post test for $k = 8$ ($p < 0.05/k$, i.e., $p < 0.000625$).

TableVI. Allele frequencies of XMEs in psoriasis patients and healthy volunteers

	Patients		Controls			
Enzyme[a]	RefS[b]	VarS[b]	RefS	VarS	p-value	OR (95%CI) of variant allele[c]
1A1 (*2C)	0.991 (618)	0.019 (12)	0.959 (441)	0.041 (19)	0.041	0.44 (0.21–0.92)
1A1 (*2A)	0.932 (585)	0.068 (43)	0.932 (431)	0.079 (37)	0.557	0.85 (0.54–1.35)
1B1	0.527 (332)	0.473 (295)	0.560 (263)	0.440 (207)	0.328	1.13 (0.89–1.44)
2C19	0.854 (545)	0.146 (93)	0.835 (392)	0.165 (76)	0.448	0.88 (0.62–1.22)
2E1	0.978 (626)	0.022 (14)	0.970 (456)	0.030 (14)	0.442	0.72 (0.34–1.54)
NQOR	0.813 (509)	0.187 (117)	0.833 (390)	0.167 (78)	0.425	1.15 (0.84–1.57)

[a]Enzymes were genotyped by RFLP-PCR for reference and variant allele.
[b]Frequency of reference allele (RefS) and variant allele (VarS) was calculated from genotyped patients assuming Hardy–Weinberg equilibrium. Absolute numbers of alleles are given in parentheses.
[c]ORs for the number of reference alleles and of variant alleles in patients and controls were calculated by Fisher's exact test. 95%CI were calculated. OR <1 indicates a protective effect associated with the respective variant allele.

Variant alleles were more common in healthy control subjects. In particular, 16 control subjects, but none of the patients, were heterozygous carriers of variant allele *CYP1A1 *2C* ($p < 0.0001$). Likewise, heterozygous carriers of *CYP1A1 *2A* were more abundant in the controls ($p = 0.0086$) (see **Table IV**). The OR for carriers of any of the two variant alleles was 0.44 ($p = 0.0004$), indicating a protective effect of this variant allele in comparison to *CYP1A1 *1A* (**Table V**).

With respect to the phase II enzymes, the analysis of carriers of the three genes *GSTM1*, *GSTT1*, and *NQOR* did not yield a significant result. ORs for *NQOR* were approximately the same, even if slightly diluted, when we evaluated the ORs for allele frequencies rather than number of carriers (compare **Table V** and **Table VI**).

Combinations of several phase I and phase II enzyme alleles have synergistic risk/protective effects Few diseases are caused by a single genetic defect; many more are multifactorial. Exploring our data further, we analyzed whether or not combinations of metabolizing enzyme variants are conspicuously prevalent in psoriasis patients compared to healthy controls. Only three combinations turned out to be of possible interest. The combination of the reference allele of *2C19 *1A* [OR = 3.3 (1.36–9.66), p = 0.012] or *GSTM1* [OR = 4.84 (1.29–18.13), p = 0.018] with *CYP1A1 *1A* is found more often in patients than in controls. The high ORs are suggestive of a susceptibility effect for this genetic background, although the 95%CI are rather broad. Note that no post test was applied due to the explorative nature of the analysis. Also, the combination of reference allele *GSTM1* and heterozygosity for *NQOR* gives an OR of 1.77 (1.02–3.07), p = 0.045.

***CYP2C19* homozygosity as a risk factor for early onset psoriasis** Psoriasis can be divided into "early onset" and "late onset" type of disease. As environmental factors may accumulate in life, they conceivably could contribute more strongly in older patients. Therefore, we tested early onset (before age 40) and late onset (above age 40) psoriatic patients for differences in XMEs. We found that *CYP2C19 *1A* homozygotic carriers are found more often in the early onset group (75%) than in the late onset group (66%); this genotype confers a risk twice as high [OR = 2.20 (1.08–3.87), p = 0.0249]. The majority of our persons tested were homozygous for *CYP2C19 *1A,* so for early onset the more common genotype is associated with risk. Or, in other words, the rarer *CYP2C19 *2A* protects from early onset of psoriasis. For none of the other phase I and phase II enzymes could a difference between early and late onset patients be detected.

As *CYP2C19* and *CYP1A1* showed as susceptibility genes for psoriasis, we tested for combinations of these genes in the early and late onset subgroups. The presence of any of the variant alleles of these two enzymes turned out to be a protective factor for early onset psoriasis [OR = 0.52 (0.30–0.91), p = 0.023] compared to late onset patients. This is significant, even after Bonferroni post testing.

***CYP2C19* heterozygosity protects from PsA** PsA is a form of the disease with involvement of the inner epithelia. Altogether, 23% of the female patients and 31% of the male patients had PsA. We tested for links of certain gene polymorphisms with PsA. Again *CYP2C19* came up. Heterozygosity for *CYP2C19 *1A*2A* appears to be a risk factor for psoriasis vulgaris: 45 patients (26%) were heterozygous, and only eight (12%) of the patients suffering also from PsA [OR = 2.5 (1.15–5.56), p = 0.024].

DISCUSSION

In a healthy person, the destructive power of autoreactive T cells is kept under control. In autoimmune diseases this immunologic tolerance is broken, and T cells play a pivotal role in the pathologic processes (Chang *et al*, 1994). Although it is not entirely clear whether psoriasis is an autoimmune disease, it has many autoimmune features, and T cells are known to be essential. One possibility to break immunologic tolerance is the generation of novel self-peptides by changes in antigen processing, e.g., via environmental agents. Chemicals or their protein-reactive metabolites can either act as haptens themselves or change the normal degradation of proteins. An increasing body of evidence supports this notion. Drugs like procainamide or propylthiouracil, chemicals like trinitrophenyl derivatives, and even metal salts have been shown to be able to break tolerance and activate T cells (reviewed in Griem *et al*, 1997). Could such a mechanism also be underlying the susceptibility to psoriasis, a disease that can be triggered by chemicals and drugs?

Susceptibility to autoimmune diseases is in part genetically determined. Psoriasis is no exception. Good associations are found with certain HLA class I and class II alleles, which is not surprising given the role of these molecules in the presentation of antigen peptides to T cells (Henseler and Christophers, 1985; Schmitt-Egenolf *et al*, 1993). The effects of chemical/environmental triggers of psoriasis can vary, suggestive of genetic factors (Baker, 2000). We look at XMEs as such possible factors.

We analyzed the genes of four polymorphic phase I XMEs and three phase II enzymes in more than 300 patients with psoriasis. Our results are in good agreement with allele frequencies reported in the literature for the various enzymes, indicating that neither patients nor control persons in our study are a biased genetic subgroup of the German population (Garte *et al*, 2001b).

The number of persons carrying any of the two rare variant alleles of *CYP1A1* for which we tested was significantly larger in the healthy control group; the OR for this effect was 0.44, indicating an almost double protection. The effect was highly significant. In other words, the reference allele *CYP1A1* **1A*, the common allele carried by the majority of Caucasians, is significantly more abundant in psoriatic patients. Moreover, when exploring combinations of enzymes more risk and protective factors became evident. Combinations of the common *CYP1A1* **1A* reference allele with *CYP2C19* **1A* or *GSTM1* gave ORs of more than 3 and 4, respectively. The p-values for these combinations below 0.05 are suggestive and results can be used in further hypothesis driven epidemiologic studies.

The *CYP1A1* **1A* reference allele codes for an enyzme of lower activity than the variant form **2C*. The other *CYP1A1* variant (**2A*), characterized by an additional *Msp* restriction site, has a higher inducibility by polyaromatic hydrocarbons. Intriguingly, in humans *CYP1A1* is not expressed in the liver, but in lung, intestine, skin, and lymphocytes (Krishna and Klotz, 1994). *CYP1A2*, which catalyzes the same type of reactions as *CYP1A1*, is absent in extrahepatic tissues. Thus, when both *CYP1A1* reference alleles are present in nonhepatic tissues, the initial oxidation of xenobiotics is lower than with the variant forms, possibly leading to accumulation of xenobiotics and conceivably a higher chance of aberrant antigen formation. A higher metabolic rate provided by the variant alleles then seems to have a protective effect as shown by our results.

Before the advent of techniques allowing different *CYP* alleles to be distinguished, a number of *in vitro* functional studies were published suggestive of a link between the activity of aryl hydrocarbon hydroxylase – the old term for CYP1A1 – and psoriasis (Chapman *et al*, 1979; Shuster *et al*, 1980; Goerz and Merk, 1992). Parallel to our findings, in these studies psoriasis was found to be associated with a lower activity/inducibility of aryl hydrocarbon hydroxylase. The data were controversial, however (Bickers *et al*, 1984), and due to the technical impossibility then to identify distinct enzymes/alleles, their interpretation was limited. Our findings provide a new angle on these data, and it would be interesting to repeat some of the functional induction assays with genetically defined patient groups.

For the autoimmune disease systemic lupus erythematosus, an association with GSTM1 null allele has been shown (Ollier *et al*, 1996). The enzymatic action of GST can yield DNA- and protein-reactive metabolites. It is tempting to speculate that in psoriasis the *GSTM1* pathway might yield protein-reactive substances that can cause generation of neoantigens. Detoxification by NQOR is accompanied by the generation of reactive oxygen species, which are known to activate macrophages, key cells in antigen presentation. The combinations of $GSTM1_{null}/NQOR_{het}$ gave an OR of 1.77 with $p = 0.04$. This is not significant when the Bonferroni statistical adjustment for multiple testing is used (in our exploration of eight enzymes giving 33 combinations $k = 33$, necessitating lowering α to 0.0015). Considering functional assays or metabolic pathways, however, these values are suggestive, and we would take these enzymes into consideration nonetheless. Xenobiotics can be metabolized not only in liver cells but also extrahepatically. There is evidence that metabolism in antigen presenting phagocytes is involved in systemic adverse immune reactions to procainamide, propylthiouracil, and gold(I) thiomalate (Kubicka-Muranyi *et al*, 1993; Goebel *et al*, 1995; von Schmiedeberg *et al*, 1996). Further functional studies in the metabolic and immunogenic pathways of psoriasis-inducing drugs would be necessary to clarify whether and how such scenarios play a role in psoriasis pathogenesis.

Our patient group showed two distinct subpopulations. In the majority of patients, disease onset of psoriasis had occurred in their teens; in the other group, onset occurred later in life, at an average age of 50. Conceivably, the latter group might have had a bigger chance of exposure to triggering factors, or accumulated xenobiotics in life. As expected, the effects of *CYP1A1* remained; however, they were exacerbated for late onset if patients were also homozygotic carriers of the *CYP2C19* **1A* reference allele. What could explain this effect? CYP2C19 is an enzyme responsible for the metabolizing of, among others, propranolol and proguanil, a beta-blocker and an antimalarial, respectively. This fits with our idea that triggering of psoriasis by such drugs might correlate with the way the body metabolizes them. The rare variant allele *CYP2C19* **2A* is associated with poor metabolic activity. Unfortunately, it was impossible to link psoriasis, genotype, and anamnestic data on drug usage over lifetime, because the memory of the patients about the drugs they had taken was incomplete. In particular, a statistically significant link to the beta-blocker propranolol could not be established, as only seven patients reported having taken this drug. Establishing such a link with an analytical epidemiologic study would be necessary to clarify whether metabolizing xenobiotics at a higher rate by CYP2C19 is a step towards the disease.

Although they have broad and overlapping substrate specificities, the K_m values of single CYP450 enzymes can dictate the limiting step in metabolizing a chemical. For instance, only CYP2C19, but not CYP3A4, metabolizes diazepam *in vivo* (Kato and Yamazoe, 1994), although both are capable of doing so *in vitro*. Eventually, it has to be determined, for each xenobiotic, which enzymes participate in metabolizing. Our explorative approach thus can only correlate genotype and risk, but is of limited value to pinpoint the culprit drugs.

Whereas heterozygosity for the *CYP2C19* alleles was a risk factor for late onset of psoriasis, the same genotype turned out to be a protective factor against PsA. In good agreement with the literature, about one-third of our patients suffered from PsA, usually in addition to psoriasis vulgaris (Scarpa *et al*, 1984). As described above, the variant *2C19* allele codes for an enzyme of lower activity. Interestingly, in Japan, where the variant isoform is about 10 times more common (approximately 12%–23%), the incidence of arthropathic psoriasis is reportedly lower than in Caucasians, only 12.7% (Hukuda *et al*, 2001).

In conclusion, we have shown that some XMEs, in particular *CYP1A1* and *CYP2C19*, can be susceptibility factors in psoriasis, adding these genes to those already considered important in the

pathomechanism of the disease (Burden *et al*, 1998; Elder *et al*, 2001). Our findings supply important background information to tackle the problem of how metabolizing xenobiotic substances can be linked to psoriasis and eventually whether environmental factors interfere with antigen presentation by the immune system. Even though our data do not have a direct therapeutic impact, they reassert the medical necessity of care in the prescription of certain drugs, like beta-blockers, to psoriatic patients (Gold *et al*, 1988).

One last caveat: as we could see in our study, a genotype associated with an increased risk for situation A (here: *CYP2C19* and late onset psoriasis) might at the same time be associated with a decreased risk for situation B (here: *CYP2C19* and PsA). Therefore, it cannot be concluded that there is an ideal genotype for all situations. This is also true for other diseases and genotypes. Genetic variation in humans is an important factor in being "prepared" for all evolutionary challenges that might yet come.

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